REMARKS

Reconsideration and withdrawal of the rejections of the application are respectfully requested in view of the remarks and amendments herewith.

I. STATUS OF THE CLAIMS AND FORMAL MATTERS

Claims 1-2, 4-12 and 15-32 are now pending, and claims 1-2, 4-12, 15-17, and 22-25 are under examination. Claims 1,23, 26 and 28 have been amended, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents.

No new matter is added.

It is submitted that these claims are in full compliance with the requirements of 35 U.S.C. §112. The amendments to the claims and the remarks herein are not made for the purpose of patentability within the meaning of 35 U.S.C. §§ 101, 102, 103 or 112; but rather the amendments and remarks are made simply to place the claims in better condition for examination and to correct typographical errors.

II. THE OBJECTIONS TO THE CLAIMS ARE OVERCOME

Claims 15-17 were objected un under 37 CFR 1.75(c) as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicants respectfully disagree.

The Office Action alleges that because claim 1 recites "a bacterial, yeast or fungal cell comprising ... recombinant POI", the cell is necessarily a transformed cell with a nucleic acid encoding a POI using recombinant DNA techniques as required by claims 15-17. Applicants respectfully assert that this reading of the claims fails to consider that it is not necessarily the case that a bacterial, yeast or fungal cell comprising a recombining POI is the same as a transformed cell. For example, a bacterial, yeast or fungal cell comprising a recombining POI could be a descendant of a transformed cell. That is, a bacterial, yeast or fungal cell comprising a recombining POI could be the progeny of a bacterial, yeast or fungal cell that was transformed with a nucleic acid encoding a POI using recombinant DNA techniques as required by claims 15-17.

Accordingly, Applicants respectfully submit that claims 15-17 are proper dependent claims, and should remain in the present application.

Claim 23 was also objected to because it recited the amino acid sequence set out in SEQ ID NO: 22 instead of SEQ ID NO: 23. Applicants respectfully submit that this correction has been made in the present amended claim set.

Therefore, for all of the reasons set forth above, reconsideration and withdrawal of the objections of the claims is respectfully requested.

III. THE REJECTIONS UNDER 35 U.S.C. §112 ARE OVERCOME

Claims 1, 2, 4-12, 15-17 and 2-25 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the invention at the time of filing. Applicants respectfully traverse.

Specifically, the Office Action indicated that the recitation "for between 4 to 48 hours" was new matter not supported by the specification. Although Applicants believe the phrase is supported, in order to further prosecution, the recitation has been removed from the claim herein.

Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 1, 2, 4-12, 15-17 and 22 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the application at the time of filing. Applicants respectfully traverse. Specifically, the Office Action alleges that the specification and claims do not indicate what distinguishing attributes are shared by the members of the genus of POIs which can be made according to the pending claims.

And, claims 1, 2, 4-12, 15-17 and 22-25 were rejected under 35 U.S.C. §112, first paragraph, because the specification is allegedly not enabling for a method of releasing any POI using an quarternary ammonium at concentration 0.05%-6.0% within 4 to 48 hours at undefined pH and temperature. Applicants respectfully traverse. The written description and enablement rejections will be addressed collectively.

35 U.S.C. §112, first paragraph, requires that the specification describe how to make and use the invention. 35 U.S.C. §112, first paragraph, recites, in pertinent part:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same[.]

A patent claim is invalid if it is not, *inter alia*, supported by an enabling disclosure. The test for enablement requires a determination of whether any person skilled in the art can make and use the invention without undue experimentation. *See In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400, (Fed. Cir. 1988). The factors involved in determining whether there is sufficient evidence to support a finding of enablement include, among others, (1) the breadth of the claims, (2) the nature of the invention, (3) the state of the prior art, (4) the level of one of ordinary skill, (5) the level of predictability in the art, (6) the amount of direction provided by the inventor, (7) the existence of working examples, and (8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *See Wands*, 858 F.2d at 737, 8 U.S.P.Q.2d at 1404.

It is respectfully submitted that the present application satisfies both the written description and enablement requirements as described above.

Initially, the Office Action alleges that the claims are drawn to a method of making a genus of POIs, for which no distinguishing attributes are provided. This allegation is simply unfounded when the claims are read in light of the specification. The claims specifically require that the POI be a soluble or membrane associated intracellular recombinant protein from a bacterial, yeast or fungal cell, wherein the protein is released from a bacterial, yeast or fungal cell. In other words, guidance is provided as to the type of protein, as well as the original source of the protein. Guidance is also provided as to the type of cell from the recombinant protein is to be extracted.

The Office Action further indicates that specific guidance is needed in regards to the purification scheme because trial and error would be necessary. Applicants respectfully submit that any such experimentation necessary to optimize the extraction process is merely routine, and would not be considered undue by one of skill in the art. Indeed, the Office Action appears to be contradictory on this point as on page 4 it is alleged that the "trial and error experimentation" required by protein purification requires Applicants to provide specific extraction schemes, while

at pages 9-10 it is stated that one of ordinary skill in the art would be try various combinations of concentrations and incubation times to choose the most suitable, and that one of skill in the art would have a reasonable expectation of success based on using the general teachings of the reference as a guide. These statements are in clear opposition to each other - and it should be clear that routine optimization is a normal practice in the art that would be expected by those of skill in the art. Simply, no undue experimentation is necessary and one of skill in the art would have no difficulty utilizing the teachings of the present invention for use with other proteins and other species of cells.

Indeed, Applicants respectfully submit that the specification as filed provides a detailed teaching of how the invention may be performed generally, for POIs expressed in a bacterial, yeast or fungal cell. Furthermore, the Examples teach that the invention may be performed across a number of different organisms, including *Chondrus crispus, Hansenula polymorpha* and *Pichia pastoris.*

The Office Action further alleges that only the extraction of HOX is illustrated in the present invention. This is an erroneous reading of the present application, which also describes the extraction of interleukin I receptor antagonist (IL-Ira) enzyme as well as glucan lyase.

In addition, the specification as filed describes in detail the components of a membrane extracting composition, as well as the conditions under which such a composition may optimally be used. The membrane extracting composition is described as containing one or more quarternary ammonium compounds (see page 13, lines 303 to 310) which are described fully on page 13, line 311 to page 17, line 368. Indeed, LTAB, CTAC and CTAB are specifically provided as examples of quarternary ammonium compounds useful in the present invention.

Furthermore, details of the amount of quarternary ammonium compound(s), temperature, and pH are provided on page 19, line 406 to page 21, line 456. The specification as filed discloses expression of proteins, including in yeast host organisms (page 26, starting at line 561) and provides detailed instructions on how to extract these expressed proteins from yeast cells, using quarternary ammonium compounds, examples of which are set out at pages 13 to 17. Again, the conditions which are required for releasing the protein from the yeast cells are discussed generally at pages 19 to 21, which describe the concentration of the quarternary ammonium compound as well as the temperature and pH at which these may be used.

Detailed experimental protocols are also set out in the Examples.

Examples 1 to 3 describe the expression of recombinant hexose oxidase (HOX) in *Hansenula polymorpha,* while Example 4 shows that the basic extraction method works. Examples 5 and 6 compare extraction using different membrane extraction compositions. Example 7 shows that protein is extracted without contaminating compounds, while Example 8 explores the effects of temperature on protein production. Examples 9, 10 and 11 compare various compounds and their effect on the extraction of protein. Examples 12, 13 and 14 demonstrate that it is possible to scale up the process. Accordingly, it is evident that protein extraction using the method described and claimed may be achieved on a commercial scale.

Example 17 shows that a number of HOX enzyme mutants may be expressed and extracted from *H. polymorpha.* Example 20 describes expression and isolation of a number of IL-1 receptor agonists, as does Example 21. The Examiner's attention is specifically directed to Tables 16 and 17, which show the extraction of these proteins. The extracted proteins are clearly seen to have higher specific activities compared to proteins extracted by mechanical means.

Examples 24 to 33 describe the expression and extraction of another protein (namely, a-l,4-glucan lyase from the yeast *H. polymorpha.* The expressed protein is extracted using LTAB, a quarternary ammonium compound, by a method as described and claimed in the present application. It is clear from these Examples that the expressed and extracted glucan lyase has a specific activity as high as 9.2 μmol 1,5 anhydrofructose/min.mg. In contrast, Yu *et al* (1999 - mentioned in the last two paragraphs of page 105) reported expression and extraction of glucan lyase by secretion by means of a signal peptide, with a low specific activity and yield of 0.7 μmol 1,5 anhydrofructose/min.mg protein. As is clearly demonstrated by these Examples, extraction by quarternary ammonium compounds of intracellularly expressed recombinant proteins therefore results in a higher yield than the prior art methods.

In summary, the Applicants have, for the first time, disclosed a method for extracting proteins from yeast, fungal and bacterial cells by exposure to a membrane extracting composition comprising a quarternary ammonium compound. With the present specification serving as a guide, the skilled person can refer to the detailed description provided in the specification to identify a precise method to extract the particular protein of interest by routine determination and optimization, which a skilled person would be fully able to do without undue burden (as is typical in the art, and which is essentially acknowledged in the Office Action at pages 9-10). Clearly, the present invention fulfills the written description guidelines by having provided

numerous examples of quarternary ammonium compounds, specific ranges of temperatures and pH levels, multiple examples of species in which the invention can be practiced, as well as the demonstration of the extraction of multiple POIs.

And, consequently, when the present invention is viewed in light of the *Wands* factors, it is clear that the breadth of the claims is sufficiently appropriate based on the method provided, the level of one of ordinary skill is high, the level of predictability in the art is high given the knowledge of this in the field and the specific guidelines provided in the specification, the amount of direction provided by the inventor is high, working examples exist in the specification and provide variations of the method within the scope of the claims, and the quantity of experimentation needed to make or use the invention based on the content of the disclosure is low as a result of the described elements, i.e., the identification of quarternary ammonium compounds, guidance as to the concentrations at which such compounds should be utilized, and guidance as to incubation times.

For all of these reasons, the presently pending claims are meet the written description requirement and are fully enabled by the specification as filed, and reconsideration and withdrawal of the written description and enablement rejections under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 1, 2, 4-12, 15-17 and 22-25 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particular point out and distinctly claim the subject matter of the invention. The rejection is respectfully traversed.

Specifically, the Office Action indicated that the characteristics "soluble or membrane associated" refer to a native protein and not to a protein obtained by recombination. Further, the Office Action stated that it was unclear whether the POI should be a bacterial, yeast or fungal protein or if it can be any protein that is expressed in the cell.

Applicants respectfully submit that the pending claims now recite the clarifying feature that the POI is released from the bacterial, yeast or fungal cell. Applicants believe such amendment fully addresses the rejection under §112, second paragraph, such that reconsideration and withdrawal of the rejection is respectfully requested.

III. THE ART REJECTIONS ARE OVERCOME

Claims 1, 2, 4-12 and 15-17 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Puri *et al.* The rejection is respectfully traversed.

The Examiner respectfully reminded that for a Section 103 rejection to be proper, there must be some prior art teaching which would have provided the necessary incentive or motivation for modifying the reference teachings to arrive at the claimed invention. *In re Laskowski*, 12 U.S.P.Q. 2d 1397, 1399 (Fed. Cir. 1989); *In re Obukowitz*, 27 U.S.P.Q. 2d 1063 (BOPAI 1993). Further, the Examiner is respectfully reminded that "obvious to try" is not the standard under 35 U.S.C. §103. *In re Fine,* 5 U.S.P.Q. 2d 1596, 1599 (Fed. Cir. 1988). And, as stated by the Court in *In re Fritch*, 23 U.S.P.Q. 2d 1780, 1783-1784 (Fed. Cir. 1992): "The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification." Also, the Examiner is additionally respectfully reminded that for the Section 103 rejection to be proper, **both the suggestion of the claimed invention and the expectation of success must be founded in the prior art, and not Applicants' disclosure**. *In re Dow*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

The Examiner is also respectfully reminded that MPEP 2143.01 mandates that for a Section 103 rejection, there must be some suggestion or motivation to modify reference teachings, and, that MPEP 2143.02 further mandates that for a section 103 rejection, there must be a reasonable expectation of success.

The Office Action alleges that Puri *et al.* teaches the method for solubilization of recombinant protein expressed in *E. coli* using CTAC and that it would have been obvious to try various combinations of CTAC concentrations and incubation time and choose the most suitable in view of various considerations.

The present invention relates to methods of extracting a soluble or membrane associated intracellular recombinant protein of interest (POI) from a bacterial, yeast or fungal cell, the POI being released from the bacterial, yeast or fungal cell comprising **contacting the cell** with a membrane extracting composition comprising a **quarternary ammonium compound**.

Applicants respectfully submit that Puri *et al.* has been misapplied to the claims as they currently stand. Puri *et al.* does not teach the solubilization of protein from *E. coli*. Rather, Puri merely describes the use of CTAC to solubilize **inclusion bodies** which have **already been**

isolated from *E. coli.* (see abstract, "Recombinant pig growth hormone (rPGH) **was solubilized from inclusion bodies** by using the cationic surfactant ... CTAC", emphasis added). Indeed, the experimental procedure described in Puri *et al.* involves **disruption** of the *E. coli* cells with **high pressure** (69000 kPa, see page 872, second paragraph: "E. coli cells ... were pelleted ... and lysed in a pressure vessel"), followed by isolation of the inclusion bodies. CTAC is then used to solubilize the isolated inclusion bodies. The art recognizes that inclusion bodies are solid in nature, and are not soluble or membrane associated as required by the claims. Furthermore, the claims require the release of the protein of interest **from the cell** by **contacting it with a quarternary ammonium compound**, which is not taught or suggested by Puri *et al.*

Indeed, in contrast to the present invention, in the method of Puri *et al.*, the inclusion bodies (containing the putative protein of interest) are released from the cell not by use of a quarternary ammonium compound (as is required by the presently pending claims), but rather by high pressure used to disrupt the *E. coli* cells. There is no teaching or suggestion anywhere in Puri *et al.* that the quarternary ammonium compounds can or should be used to solubilize anything other than the inclusion bodies.

There is also no disclosure or suggestion that the quarternary ammonium compounds can be used to release already soluble proteins. Indeed, Puri *et al* actually teaches away from the present invention. A skilled person reading Puri *et al* and seeking to solve the problem of extracting a soluble or membrane associated protein would not consider releasing the protein from the cell with a quarternary ammonium compound and recovering it from the composition. Rather, Puri *et al.* teaches that it would be more effective to cause the soluble protein to form inclusion bodies (for example by altering the conditions of the cell culture), and then to extract the inclusion bodies from the *E. coli* by pressure and solubilising the inclusion bodies with a quarternary ammonium compound. Nothing in Puri *et al.* teaches or suggests that one of skill in the art modify the method of Puri *et al.* to forgo the creation and release of inclusion bodies and to instead solubilize the *E. coli* directly instead of the inclusion bodies, as is required by the present claims which recite that the **POI is released from the bacterial, yeast or fungal cell**. Clearly, Puri *et al* teaches away from the invention as claimed.

Therefore, as Puri *et al.* neither teaches or suggests each and every element of the claims, nor does Puri *et al.* provide any motivation for one of skill in the art to modify the teachings of Puri *et al.* (indeed, Puri *et al.* teaches away from the present invention), the rejection cannot

stand.

Consequently, reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

REQUEST FOR INTERVIEW

If any issue remains as an impediment to allowance, prior to issuance of any paper other than a Notice of Allowance, an interview, is respectfully requested, with the Examiner, her supervisor, and, the Examiner is respectfully requested to contact the undersigned to arrange a mutually convenient time and manner for such an interview.

CONCLUSION

In view of the amendments, and remarks herein, the application is in condition for allowance. Reconsideration and withdrawal of the rejections of the application, and prompt issuance of a Notice of Allowance, is respectfully requested.

Respectfully submitted,
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